Investigating skin barrier function utilizing reflectance NIR Spectroscopy

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***Abstract*— Near Infrared Spectroscopy is seen as a potentially valuable technique for skin analysis, and has been employed by many previous studies to measure skin hydration, since it is competent of providing information regarding various functional groups including OH, CH and NH bands. The aim of this study was to investigate the capability of further utilizing this method by attempting to analyze skin barrier function as well as water content, through the evaluation of skin water uptake on two test sites, one untreated, and another treated with a high lipid moisturizer for a period of 7 days. Reflectance NIRS measurements were supported by capacitance readings obtained using the Corneometer® CM 825. Baseline recordings taken on the first day following treatment showed that more differences were observed between the treated and untreated sites in the regions belonging to, or are influenced by CH and NH groups rather than purely on the water bands. On the hand, moisture levels measured after placing a wet patch on the skin remained nearly equal for both sites but second derivative spectra showed that a clear contrast existed between absorbance heights at the water bands of the treated and untreated, suggesting that moisturizer use could have limited water uptake to a more superficial layer of the skin, whereas for the untreated site, the opposite would have been true and water was able to penetrate deeper. Overall, results here suggest that NIR spectroscopy can possibly provide valuable information not only on skin water contents but perhaps on other skin parameters such as barrier function.**

I. Introduction

Skin barrier function and hydration are some of the most commonly measured parameters in skin research and health [1], either for pathological or cosmetic purposes. The two are considerably interrelated as the skins' barrier function performs the vital task of preventing the entry of exogenous toxic/chemicals into the body and excessive losses of internal body components, whereas the skin's water content allows it to maintain its flexibility and facilitates the occurrence of necessary enzymatic reactions inside the skin [2].

For *in vivo* measurements, these parameters are normally assessed through the use of the "open chamber" technique to obtain transepidermal water loss (TEWL) readings to monitor skin barrier function, or through the use of electrical capacitance methods for evaluations of skin moisture content [3].

Alternatively, Near Infrared Spectroscopy (NIRS) is emerging as a potentially valuable technique for skin analysis, as many previous studies have employed it to study skin hydration and water content [4–7], as well as characterization of skin properties [8]. Combined with suitable statistical methods, NIRS has shown to be capable of direct and accurate detection of water inside the skin and evaluation of skin water types, using the overtone and combinations bands of water, and of providing additional information regarding other skin constituents such as lipids and proteins [4–6]. Therefore, the aim of this study was to further examine skin assessment possibilities of NIRS by investigating whether skin barrier function can be determined. This will complement our previous work on skin water content measurements using this method and future aim of developing a novel optical device that is based on the principle of NIRS [9-10]. To do this, we have looked at the works of recent studies focused on determining the effects of long term moisturizer application on normal skin. According to such studies [11], long-term use of certain moisturizers for cosmetic purposes can weaken skin barrier function, as well as enhance its susceptibility to irritants and influence skin barrier recovery. However, unlike these studies which investigated this effect through measuring the skins' response to irritants, it was instead decided to examine the skin's uptake of water applied on the skin, as water itself can act as a mild irritant, and prolonged skin contact with extrinsic water is known to be innocuous, which can possibly lead to Stratum Corneum (SC) swelling, increase in SC suppleness, weakening SC corneocyte cohesion, and increase the permeability [12]. Thus, it was assumed here that the depth of water travel inside the skin would be an indication of its permeability capacity, and hence its barrier function capability in preventing substances penetrating the skin.

As electrical-based methods are the current gold-standard instruments in skin hydration measurements, capacitance readings were also included here in order to support results from reflectance spectra, which were recorded with a spectrophotometer operating in the NIR region and equipped with a customized fibre optic probe to allow *in vivo* recordings.

II. Materials and Methods

This study was approved by the Senate Research Ethics committee at City University London prior to performing any measurements on individual(s) or collecting any personal details.

Research supported by City University London.

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Seven participants with no history of skin pathology and various skin complexions (male: 4, female: 3, age range: 21-42 years old, mean age: 27.4 years) were enrolled into the study after completing a skin assessment questionnaire and giving written informed consent. All panelists were asked to refrain from applying any moisturizer on the day starting the experiment, and had not shaved, waxed or used laser hair removal since at least a week before.

A. Experimental Design

To begin with, a square area of 25 mm^2 was drawn on the interior of both forearms of each volunteer to mark the test site.

Since volunteers had already refrained from using any product(s) on their forearms on the day attending the experimental session, further skin preparation was not necessary. Baseline measurements were recorded by placing the Corneometer® CM 825 probe on the marked test site of the right forearm, taking three readings from within the marked square area. Then, the right forearm was placed underneath a fibre optic probe clamped onto a stand and connected to a spectrophotometer to allow recordings of NIR skin spectra. Again, three spectra were recorded for averaging purposes. Next, an untreated bandage cloth previously immersed in water was placed on the test area on the right forearm for 15 minutes before it was removed. Immediately after removing the patch, the skin was slightly patted with paper towel to remove residues on the surface, and the same measurements of skin capacitance and NIR scans were repeated on the marked site.

The same procedure was also repeated for the second test site on the left forearm. Then, participants were each given a common, high lipid content moisturizer to use twice a day on one of their forearms for a period of 7 days without any other product, whilst the second forearm was to serve as a control, and so volunteers refrained from applying any moisturizer or oils for the entire study period. Follow-up measurements were performed on the first post-treatment day, and the exact protocol of data collection outlined above was repeated.

B. Instrumentation

Skin capacitance measurements were performed using the Corneometer® 825 (Courage-Khazaka electronic GmbH, Koln, Germany) whose output is an arbitrary number deduced by determining the change in the dielectric constant due to skin surface hydration changing the capacitance output. All values obtained were then transferred to Excel (Microsoft Office) for further processing.

As for the NIR skin spectra, these were collected using the Lambda 1050 dual beam UV/Vis/NIR spectrophotometer (Perkin Elmer Corp, Massachusetts, USA) at increments of 2 nm in the spectral region of 900-2100 nm, with an InGaAs detector operating throughout the entire region. Light was provided by a tungsten lamp and a gain setting of 3 was also added to measure the energy in the single mode beam and give better quality spectra. The scanning period for each interval was set at 0.2 secs.

Attenuator settings were kept constant at 1% for the reference beam to improve noise levels at high absorbance, and at 100% attenuation for the sample beam. Slit size controls for the InGaAs detector which allow one to adjust the amount of light entering was set on "servo mode" so that the system could oversee the reference beam energy and select the slit size accordingly.

Initial baseline corrections were performed to eliminate irrelevant bands and background noise especially evident in highly absorbing media such as skin. These corrections were carried out at 100 % T/0A baseline to correct maximum sample and reference beams, and at 0 % T/blocked beam to regulate the beams at 0 % for the sample and 100 % for the reference.

To allow reflectance measurements, it was necessary to replace the sample holder compartment with a reflectance accessory that permitted the attachment of a fibre optic probe (Ocean optics, Duiven, The Netherlands). The fibre core diameter was 600 μm, allowing a reasonable area of skin to be sampled.

It was essential to introduce a small gap between the probe and the test site to prevent blockage of light leaving and entering the probe, and also since occlusion can lead to build up of water and increase hydration, thereby falsely raising skin water content measurements. Therefore, the reflectance probe was slightly modified by enclosing its tip with a Perspex tube layer that was longer than the end of the probe by 1.5 mm. This coating ensured that the desired separation distance was maintained throughout all tests. In addition, this particular coating was overlaid with another white coating to eliminate interference from ambient light whilst scanning.

NIR spectra obtained were pre-treated using the UVWinlab Data Processor and Viewer software (Perkin Elmer Corp, Massachusetts, USA), where smoothing, R% to Absorbance conversion and Standard Normal Variate (SNV) scatter correction were applied. Further data analysis was later finalized using the Matlab software (Mathworks Inc., Novi, MI).

III. RESULTS AND DISCUSSION

Reflectance spectra obtained throughout the study were firstly transformed into apparent absorbance using the equation Log 1/R, before being pre-treated using the block average method for smoothing and Standard Normal Variate (SNV) for scatter correction. To begin with, baseline spectra recorded on the 0^{th} and 8^{th} day of the study were compared between the treated and untreated test sites, and are shown in Fig 1. This figure shows that the most apparent changes that occurred between the treated and untreated test sites after the 7 day testing period were most visible in the combination bands of water near 1450 and 1900 nm, respectively, although in both cases, a decrease is seen in both regions. Moreover, as the water band around 1900 nm decreased, other bands in the region between 1850-2040 nm became more prominent and less resolved. Nevertheless, the untreated site seemed to show more prominent features and slightly smaller decreases at 1450 and 1900 nm. The main difference between the treated and untreated sites measured

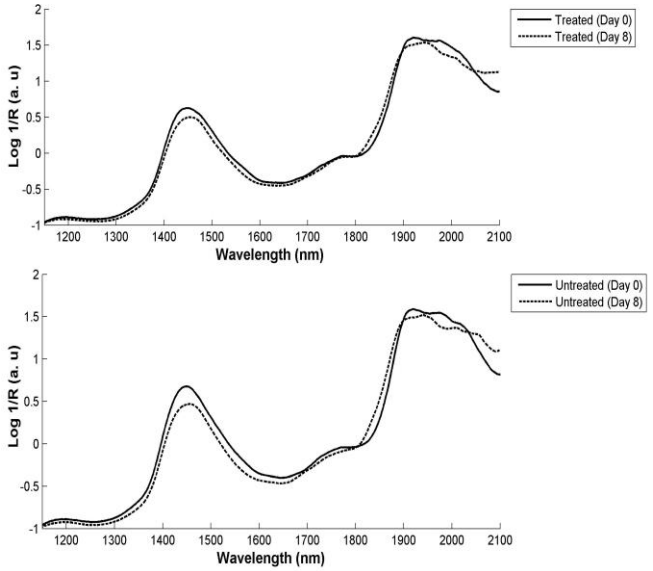

Figure 1. Averaged NIR Apparent absorption spectra of baseline measurements taken from both the treated and untreated forearm, and on Day 0 and Day 8 of the experiment.

on the 8th day were seen at the region between 1700-1800 nm belonging to CH vibrations. Unlike the site treated with moisturizer, a decrease was seen in the untreated forearm. This leads to the assumption that consistent moisturization mainly effected the CH bands in skin, especially since the reduction seen for the untreated site at the 1450 nm band was more significant, and this particular band is in fact influenced by adjacent protein bands at 1400 and 1500 nm resulting from the combination of CH stretching and CH bending modes, and the first NH2 overtone, respectively.

This point was further clarified by calculating the difference between the baseline measurement taken on day 0 and day 8 for the treated site and the same for the untreated. The resulting difference spectra of this is shown here in Fig 2, where it can be seen that divergences between the two sites were minimal at the 1900 nm, and contrarily to the untreated forearm, the treated one showed an increase in the CH region between 1700-1800 nm, and less overall reduction.

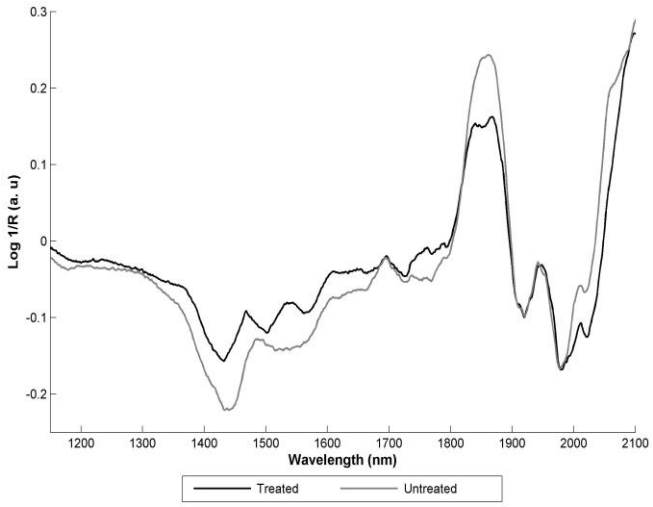

Figure 2. Difference in absorption spectra between treated and untreated test sites.

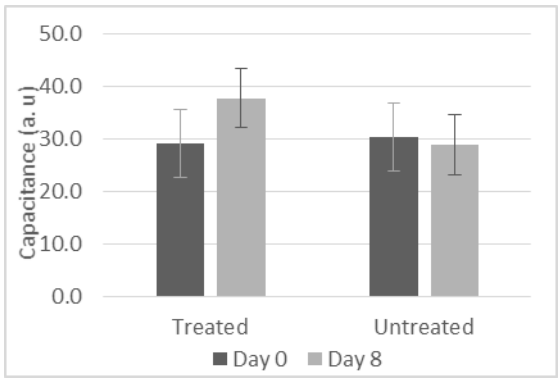

Figure 3. Chart showing mean skin capacitance values obtained from measuring the baseline of each volunteer on the 0th and 8th day of the experiment.

As for readings of skin capacitance obtained using the Corneometer®, these consistently showed an increase in skin moisture after use of the moisturizer for 7 days, as shown in Fig 3. Nonetheless, results from the untreated site were not significantly lower, and the standard deviation of these measurements, calculated for days 0 and 8, was greater for readings from day 8, although this was expected, as use of the cream would have resulted in larger changes in skin moisture.

The second derivatives of all spectra collected were also calculated using the Savitzky-Golay method. Fig 4 shows the average of these for, a) baseline recordings, and b) those taken after placing the wet patch. In both sets (Fig 4 a&b), a clear shift is observed in the 1900 nm for both spectra recorded on the 8th day, but this was higher for the treated site. According to previous studies [6-7], a peak shift at this band towards shorter wavelengths is usually indicative of increased water mobility, suggesting in this case that the treated sight had higher water mobility and thus higher water

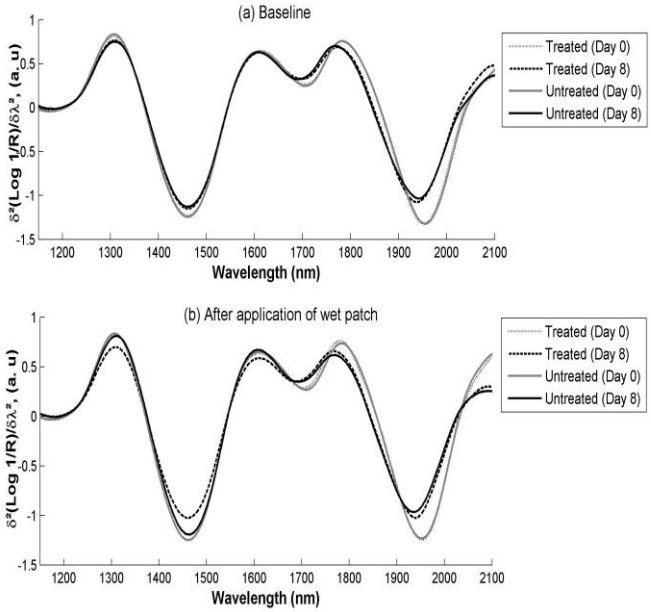

Figure 4. Second derivative spectra from untreated and treated forearms at days 0 and 8 at a) baseline, and, b) after placing a wet patch on the skin for 15 minutes.

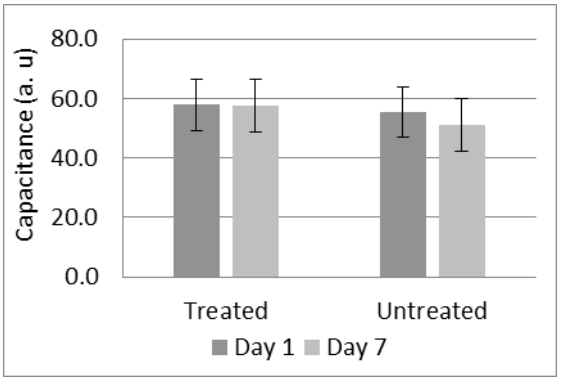

Figure 5. Chart showing mean skin capacitance readings taking after placing a wet patch on the skin for both the treated and untreated forearms, and on both the 0th and 8th day of the experiment.

contents in comparison to the untreated site, though both of these were less in minima height in relation to spectra recorded on day 0.

As for the spectra collected after placing the wet patch, shown in Fig 4(b), the mean spectrum of the untreated site shows a larger absorbance at the minima equivalent of the 1450 nm water absorption band, and less at the minima equivalent of the 1900 nm combination band. On the other hand, the mean spectrum of the treated site shows the opposite, where a higher absorbance is observed at the 1450 nm band and a smaller one at the 1900 nm band. Considering that literature [13] has shown that the light beam of the spectrophotometer would have travelled deeper into the skin at the shorter wavelength i.e. 1450 nm, it can be assumed here that the for the untreated site, the water absorbed from the wet patch had travelled deeper into the skin, whereas for the treated forearm, water absorption remained at a more superficial skin level. This point is further supported by capacitance readings as these showed almost negligible differences in skin moisture measured on both sites and on both days. These results are shown here in Fig 5, which also shows that the standard deviation between days 0 and 8 were nearly equal and without significant differences.

IV. CONCLUSIONS

A preliminary study was conducted to investigate the possibility of using NIR spectroscopy to study changes in skin barrier function as well as to provide information on the state of skin hydration. The study required volunteers to use a high lipid content moisturizer twice a day on one of their forearms for a period of 7 days, whereas the second forearm served as a control. Results from baseline measurements showed that for both treated and untreated forearms most of the difference occurred in regions where CH bands were available, although both sites generally showed alterations in water bands when compared to spectra recorded prior to the start of treatment. For spectra collected after placing a wet patch, both the treated and untreated sites yielded similar results on both days of testing, as confirmed by Corneometer® readings. However, the untreated site showed higher absorbance at the 1450 nm band and a smaller one around 1900 nm, whereas the opposite was exactly true for the treated site. Since the light beam would have travelled deeper into the skin at the shorter wavelength, it is possible to assume at this stage that long term moisturizer limited the water from deeper penetration, and that a higher concentration of the water remained at more superficial levels of the skin. To conclude, these preliminary results suggest that NIR spectroscopy can provide valuable information not only on skin water contents but perhaps on other skin parameters such as barrier function. Future work will continue to build on the results of this study and towards the design and development of a novel handheld optical device for skin measurements.

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